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Application of optical action potentials in human induced pluripotent stem cells-derived cardiomyocytes to predict drug-induced cardiac arrhythmias

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ABSTRACT

Introduction: Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are emerging as new and human-relevant source *in vitro* model for cardiac safety assessment that allow us to investigate a set of 20 reference drugs for predicting cardiac arrhythmogenic liability using optical action potential (oAP) assay.
Methods: Here, we describe our examination of the oAP measurement using a voltage sensitive dye (Di-4-ANEPPS) to predict adverse compound effects using hiPS-CMs and 20 cardioactive reference compounds. Fluorescence signals were digitized at 10 kHz and the records subsequently analyzed off-line. Cells were exposed to 30 min incubation to vehicle or compound (n = 5/dose, 4 doses/compound) that were blinded to the investigating laboratory. Action potential parameters were measured, including rise time (T_{rise}) of the optical action potential duration (oAPD).
Results: Significant effects on oAPD were sensitively detected with 11 QT-prolonging drugs, while oAPD shortening was observed with I_{Ca}-antagonists, I_{Kr}-activator or ATP-sensitive K^+ channel (K_{ATP})-opener. Additionally, the assay detected varied effects induced by 6 different sodium channel blockers. The detection threshold for these drug effects was at or below the published values of free effective therapeutic plasma levels or effective concentrations by other studies.
Discussion: The results of this blinded study indicate that OAP is a sensitive method to accurately detect drug-induced effects (*i.e.*, duration/QT-prolongation, shortening, beat rate, and incidence of early after depolarizations) in hiPS-CMs; therefore, this technique will potentially be useful in predicting drug-induced arrhythmogenic liabilities in early de-risking within the drug discovery phase.

1. Introduction

The current ICH S7B guidelines (ICH S7B and E14) were introduced and developed following the withdrawal of drugs from the market for causing QT-prolongation and Torsade de Pointes (TdP), which is primarily the result of blockade of the cardiac hERG current (I_{kr}) (Food and Drug Administration HHS, 2005a, 2005b). While the current preclinical safety assessment approaches in most pharmaceutical companies provide sufficient indications of risk (including the hERG assay), the process is often complex and incorporates many *in vitro* and *in vivo* assays involving preparations coming from multiple animal species; results of which are not always fully translatable to humans. Testing for hERG channel inhibition and QT prolongation has potentially prevented TdP-inducing new medicines from entering the market. However, this approach may inappropriately stop potential QT prolonging drugs, without overt TdP proarrhythmic risks reaching the market

Abbreviations: oAPDc, rate corrected action potential duration; CiPA, Comprehensive *in vitro* Proarrhythmia Assay; $oAPD_{50}$ and $oAPD_{90}$, 50% and 90% duration of the optical action potential duration; EADs, early after depolarizations; hiPS-CMs, Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes; ICH S7B, International Committee on Harmonisation Guideline S7B; I_{Na}, sodium current; I_{Ca}, calcium currents; I_f, hyperpolarization activated pacemaker current; I_{KATP}, ATP-sensitive potassium channel (or K_{ATP} channel); I_{Kr}, rapid delayed rectifier K^+ current; I_K, slow delayed rectifier K^+ current; I_{K1}, inward-rectifier K + channel; OAP, optical action potential; QTc, rate corrected QT-interval; T_{rise}, rise time between 10% and 90% of the AP amplitude; VSD, voltage sensitive dyes

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(Stockbridge, Morganroth, Shah, & Garnett, 2013). Conversely, the ICH S7B mandated studies may not always be sufficient to detect the proarrhythmic potential of drugs (Lu et al., 2008) (Lu et al., 2010), leading safety pharmacology departments involved in drug discovery de-risking to adopt more wide-ranging testing before first-in-human trials (Bowes et al., 2012). Drugs with multichannel effects such as verapamil and ranolazine are indeed hERG blockers, but also have other ion channel activities leading to a good balance for cardiac repolarization without potential risks for overt induction of TdP. This inadvertent triage (phenobarbital, ranolazine and alfuzosin) of potentially beneficial medicines has been the motivation for the introduction of a new Comprehensive *in vitro* Proarrhythmia Assay (CiPA), in which human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are utilized as a part of the CiPA Consortium (Sager, Gintant, Turner, Pettit, & Stockbridge, 2014) to help detect proarrhythmic risk. Furthermore, with an increasing focus on the 3R's (Replace, Reduce, Refine) in relation to animal studies, this research was undertaken to assess whether we could simplify the current *in vitro* approach utilising a human-based model to prioritize compounds in hit to lead/lead optimization, before animal studies are initiated.

As human stem cell technology can now provide a renewable and scalable source of human cardiomyocytes, hiPS-CMs are increasingly used as a new source of cardiac cells for basic research, phenotypic screening, drug safety, and toxicity assessment. These cells recreate many of the features of human cardiac physiology and can be used efficiently at a much earlier de-risking phase. In particular, hiPS-CMs fit within the current ongoing efforts of the CiPA consortium to introduce more predictive technologies for preclinical cardiac safety assessments (Cavero & Holzgrefe, 2015; Sager et al., 2014) and are generally deemed to be the future human-based cell model for detecting drug-induced long QT and cardiac arrhythmias such as TdP. To date, the following technologies have been used to detect drug-induced long QT and arrhythmias in hiPS-CM cultures: impedance-based technology (Guo et al., 2013; Liu et al., 2012), microelectrode array (Braam et al., 2013; Clements & Thomas, 2014; Harris et al., 2013; Navarrete et al., 2013), Ca^{2+} transient assay (Lu et al., 2015), and voltage-sensing-optical platforms (Klimas et al., 2016).

Voltage-sensitive dye-based optical recording is a highly efficient system to measure action potentials (AP) and is one of the key technologies currently being evaluated in the CiPA consortium. Recordings from hiPS-CMs were previously published by several groups (Khan et al., 2015; Leyton-Mange et al., 2014; Ren et al., 2011). In the present study, we investigated 20 reference drugs known to have cardiac effects using an intermediate throughput voltage-sensitive dye screening method and hiPS-CMs to determine if this technique would be reliable as a cardiac de-risking strategy for novel, lead compounds in discovery. Furthermore, to gain a better understanding of this OAP hiPS-CM model and understand if hiPS-CMs resemble the electrophysiology of human adult ventricular cardiomyocytes, we also compared the transcriptional expression of major cardiac ion channel genes between hiPS-CMs and adult human ventricular tissues.

Additionally, although the prolongation of oAPD is associated with increased risk of early after depolarizations (EADs) or TdP, the difficulty in interpretation of changes in oAPD alone is challenging since most drugs that induce changes in oAPD also change beating rate. The prolongation or shortening of oAPD is partially dependent on the change in the beat rate. Currently, formulae to correct oAPD for changes in rate are based on *in vivo* QT-correction formulae, which may not be relevant to the electrophysiological response of hiPS-CMs as these *in vivo* rate corrections may not correct appropriately through a range of, often low, beating rates observed *in vitro*. The present study measures specifically the rate dependence of oAPD in hiPS-CM to compensate more accurately for rate changes in hiPS-CMs. Thus, the present study investigates the utility of voltage-sensitive dyes to detect cardiotoxicity of novel compounds and aims to devise an accurate rate correction formula for use in hiPS-CMs.

2. Methods

2.1. hiPS-derived cardiomyocyte cell culture

Cryopreserved hiPS-derived cardiomyocytes (iCells®, CMC-100-110-001, Cellular Dynamics International (CDI), Madison, WI, USA) were kept in liquid nitrogen until cultured according to the manufacturer's instructions. Briefly, the cryovial containing iCell cardiomyocytes was thawed in a water bath at 37 °C (4 min) and then transferred to a 50 mL conical tube. The vial was rinsed with 1 mL of Plating Media (CMM-100-110-001) added drop-wise to the cells, then 8 mL of plating media were added on top. The viable cells were counted using the trypan blue (TB) exclusion method (Cell:TB at 1:1), and the cell density was calculated considering the viable cells, dilution factor, and plating efficiency (provided by manufacturer for each batch). The cells were plated at 125,000 cells/cm^2 (40,000 cells/well) in 96-well glass bottomed plates (MatTek, Cat.No. P96G-1.5-5-F) coated with fibronectin (70 μL/well at 10 μg/mL in PBS (+ Ca^{2+} + Mg^{2+}); Sigma F-1141) and maintained in a CO_2 incubator (37 °C, 5% CO_2, water-saturated air atmosphere). The first media change was made 48 h after plating, replacing the iCell Plating Media with iCell Maintenance Media (CMC-100-120-001); afterwards the cells were fed every two days. The experiments were done between Days 10–15 when the cells were a well coupled monolayer exhibiting synchronous beating.

2.2. Electrical activity measurements in hiPS-derived cardiomyocytes using voltage sensitive dyes (VSD)

Human iPS-CMs were transiently loaded with 6 μM di-4-ANEPPS (Biotium Ref. 61010) in serum-free media (DMEM Gibco #11966, 10 mM Galactose, 1 mM Na-Pyruvate). The media containing the dye was replaced by fresh serum free media and kept in this condition throughout the experiment. The cells were kept in the incubator for at least 2 h to reach a steady state before starting an experiment. The multi-well plate was placed on the stage incubator (37 °C, 5% CO_2, water-saturated air atmosphere) of the CellOPTIQ® platform (Clyde Biosciences Ltd., Glasgow, Scotland) and the spontaneous electrical activity was recorded from the di-4-ANEPPS fluorescence signal from areas (0.2 × 0.2 mm) of hiPS-CMs in individual wells visualized using a 40 × (NA0.6) objective. The electrical activity was recorded under baseline conditions for 15 s (before adding drugs or vehicle) and 30 min after drug/vehicle addition. The drugs were tested using a dose range of 5 concentrations (n = 5 for each concentration) with matched vehicle controls for each dosage. Fluorescence ratiometric signals were digitized at 10 KHz and the records were subsequently analyzed off-line using proprietary software (CellOPTIQ®). The following average parameters were obtained from the AP complexes occurring within the 15 s recording period: cycle length; rise time between 10% and 90% of the AP amplitude (T_{rise}), and action potential duration (oAPD) at 50%, 75%, and 90% repolarization. The incidence of EADs was also quantified by manually identifying and EAD like event as a positive deflection in Di-4-ANEPPS signal during the plateau phase of the oAP signal. As shown previously (Knisley, Justice, Kong, & Johnson, 2000), the ratiometric measurement of voltage using Di-4-ANEPPS generates a signal that is linearly related to transmembrane voltage making all timings and durations equivalent to direct voltage measurements. Recordings were limited to 10–15 s to collect approximately 10 oAP complexes before and after drug addition to allow comparison of drug action. Continuous recordings are possible but the serial (not parallel) nature of the acquisition requires a series of 15 s samples to be taken from each well. The absolute ratio value of the oAP signal was not used in analysis as values varied from well-to-well and within different areas within one well.

Rate corrected AP durations based on common QTc formulas (as listed subsequently) were explored to investigate the relationship between the rate and oAPD changes. These four rate correction

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